REMARKS

Claims 1-3, 5, 6, and 9-13 are currently pending in the present application. Claims 1, 5 and 9 are amended and claims 3 and 11 are canceled. No new matter has been added herewith.

Rejection under 35 U.S.C. § 103

Claims 1-3, 5, 6 and 9-13 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Lewis *et al.* (U.S. Patent No. 5,521,082) in view of Houghton *et al.* (U.S. Patent 5,350,671) and Baumert *et al.* 1998 *J Virol* **72**:3827-3836. In particular, the Examiner asserts that one would have been motivated to use Lewis' method to isolate Baumert's infection-defective HCV structural proteins and that one would have a reasonable expectation of success in isolating the infection-defective HCV structural proteins from cells infected with baculovirus encoding and expressing HCV structural proteins by precipitation with polyethylene glycol, because polyethylene glycol has been known and used in the art for the precipitation and isolation of viral proteins from virus infected cells as evidenced by Lewis and Houghton.

Lewis *et al.* teaches a method of isolating infection-defective hepatitis A virus (HAV) structural proteins form cells infected with HAV. However, hepatitis C virus (HCV) and hepatitis A virus (HAV) are very different viruses. On the one hand, HCV is an enveloped flavivirus, whereas HAV is a non-enveloped picornavirus. Enveloped viruses are well known to be more fragile; and the function of their envelope proteins is more vulnerable to stresses associated with purification procedures. PEG is well known to precipitate proteins and it allowed Lewis *et al.* to precipitate HAV. However, given the more fragile nature of enveloped HCV, one of skill in the art would not necessarily have expected PEG precipitation to result in the purification of HCV structural protein complexes or HCV-like particles.

Regarding the work of Houghton *et al.*, virions were obtained from hepatitis C patients or tentatively produced in cultured hepatocytes. Such virions are associated with lipoproteins or immunoglobulins, which impart different properties to the virions. Thus, it was not obvious that using PEG would have allowed recovery of functional HCV Env proteins from cells infected with baculovirus encoding HCV structural proteins.

Baumert *et al.* used a combination of detergent and sonication to lyse cells. When digitonin is used to permeabilize cells, it forms pores in the plasma membranes while leaving

internal cell structures mostly intact. Thus, digitonin alone is not expected to release viral particles that are embedded in endoplasmic reticulum-derived membranes. To solve this problem, Baumert *et al.* (1998) also used sonication. Since insect Sf9 cells have low cholesterol content, a high concentration of digitonin would be needed to permeabilize them. Cycles of hypertonic and hypotonic shock had previously been shown to cause disruption of the plasma membrane leading to cell lysis. However, this method was mostly used for preparing intact intracellular organelles and/or membrane fractions. It had not been shown that the method could release virions embedded in an intracellular membraneous web or vesicles, yielding functional hepatitis C virus (HCV) particles. One of skill in the art would have expected that HCV virus particles would remain associated with and embedded in intracellular membraneous vesicles.

To clarify the difference between the presently claimed method and the cited references, the Applicants have amended claim 1 by incorporating the limitation of Claim 3. Moreover, Claims 1, 5 and 9 are amended to include the negative limitation "wherein the cells are lysed without sonication". The amended claims are not obvious in light of the cited references since the references provide no reason to omit the step of sonication.

At a concentration of 0.25% or lower under conditions of low osmolarity and on ice, both of which conditions affect digitonin critical micellar concentration, digitonin acts more like a chelator of cholesterol, a lipid known to interact with HCV envelope proteins. Under these conditions, digitonin acts to facilitate HCV particle release from membranes, rather than simply permeabilizing cell membranes. Since cholesterol normally increases the interaction of HCV virus particles with membranes, chelating cholesterol with digitonin under these circumstances allows HCV particles to become released from membranes. The applicants used low concentration digitonin at the hypotonic step to take advantage of this property. In addition, the combination of low digitonin concentration and glycerol preserves the function of the purified HCV envelope proteins. The cited references provided no reason for combining both hypertonic and hypotonic shock and low concentration of digitonin without sonication to release functional HCV particles from HCV-recombinant-baculovirus-infected cells. Thus, the presently claimed methods are not obvious in light of the prior art.

In light of the amendments to the claims and the remarks above, the Applicants respectfully request removal of the rejection under 35 U.S.C. § 103(a).

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

In view of Applicants' amendments to the Specification and the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: 8 May 2008

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